THINK INDIA Journal

ISSN:0971-1260
Vol-22-Issue-16-August-2019

Occurrence of Urinary Tract Infections In Diabetic Patients: A Report From A Tertiary Care Hospital

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Abstract

This study suggested that there is an association between diabetes and risk of infections in urinary tract. We diagnosed UTIs in the diabetic patients and also isolated, identified and analyzed the susceptibility of UTI causing bacteria against various antibiotics. Overall 320 patients were diagnosed, 142 (44.4%) were found diabetic and 178 (55.6%) were non-diabetic. Bacteriuria was found in 148 (46.25%) patients out of 320 samples. The prevalence of UTIs in diabetic patients was 53.5% and in non-diabetic patients was 40.4% ($p<0.05$). Meanwhile the occurrence of UTIs was significantly ($p<0.05$) more in women than men irrespective of their diabetic status. Most of the diabetic patients were between the age group of 50 to 59 years and the most common bacterium isolated from urine samples was *Escherichia coli* (57.4%) with extended-spectrum beta-lactamases, followed by *Klebsiella* sp. (13%). The prevalence of *Candida* sp. was also observed higher in the diabetic patients than non-diabetics. UTI causing Gram negative bacteria were susceptible to colistin (100%), ceftriaxone EDTA sulbactam (100%), imipenem (86.6%), ertapenem (86.9%) and piperacillin-tazobactam (84.9%). On the other hand Gram positive *Enterococcus* was inhibited (50%) by the vancomycin, nitrofurantoin and 100% by the linzolid.

Key words: Diabeties, UTI, *Escherichia coli*, bacteriuria and antibiotic susceptibility

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Introduction

Diabetes mellitus is considered to be one of the most serious health problems of the 21st century worldwide. Around 463 million people are living with diabetes all over the world and this number will rise to 700 million by 2045. [1]. The highest number of diabetic cases (~114 million) was reported in China in 2017, followed by India (~73 million). In most countries, the proportion of people with type 2 diabetes is increasing. The rapid increase in diabetes in the Indian middle class, associated with inactive lifestyles and a shift in dietary patterns, has resulted in the current burden of this disease. [2]. Diabetes is considered a chronic disease in which a person has high blood glucose (hyperglycemia) because either the pancreas does not produce the necessary amount of insulin (hormone released by the pancreas) or the body does not respond properly to the hormone. Diabetic patients are more likely to have different types of disease than non-diabetic patients. This high prevalence rate of infection is attributed to altered immune functions such as polymorphonuclear leukocyte activity, phagocytosis, chemotaxis, and compromised antioxidant system. [3,4]. These types of alterations are responsible for making them more prone to various infections such as urinary tract infections (UTIs). Patients with diabetes have a much higher risk of UTI compared to non-diabetic patients. [5]. High sugar is the main reason for the occurrence of UTI among diabetic patients. [6]. Symptoms of UTIs such as burning sensations when urinating, fever, itching, and the occurrence of wounds in the genital area usually depend on the age and location of the infection. [7]. The most common bacterial species that are involved in UTIs are *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella* sp., *Enterobacter* sp. and *Proteus mirabilis* [8]. To date, there are no accurate data on the causes of urinary tract infections in the rural area of Punjab, India. The research was therefore performed to determine the factors associated with UTIs and their resistance to antibiotics.

Materials and Methods

Sample collection

A total of 320 samples of urine; 141 samples from diabetic patients and 179 samples from non-diabetic persons aged 21 to 90 years were obtained from tertiary hospital in Jalandhar, Punjab.

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15-20 ml of the midstream urine sample was obtained in a sterilized universal wide mouth container. Urine samples are preserved at 2–8 ° C until further use.

Health wise status of the studied population

Diabetic status of patients was observed through blood and urinary glucose levels analysis and glycated hemoglobin A1c during hospital admission. Diabetic patients (Type 1 or 2) with a fasting blood glucose level of ≥126 mg / dl or HbA1c of more than 6.1 percent were included in this study. Patients with metabolic dysfunction of the urinary tract of the kidney, malignancies, leukemia HIV, initiation of antibiotic therapy, pregnancy, congenital / acquired urogenital deficiency with recurrent catheterization have been exempted.

Physical examination of samples

Physical characteristics such as color, turbidity, odor, pH and density of the urine have been investigated. The specimens were evaluated for the presence of pus, yeast and red blood cells.

Isolation of bacterial colonies

Samples were cultured using blood agar, CLED agar and MacConkey agar (HiMedia, India) by spread-plate process and incubated aerobically at 37 ° C and examined for visible growth after 24 and 48 hr. CLED agar enhances the growth of uro pathogens and contaminants but inhibits the growth of *Proteus* species because of lack of electrolytes in the media. After the incubation, bacterial colonies were examined for their shape, size and color. On the basis of morphological and culture characteristics, most diverse bacterial colonies were further purified by streak plate method.

Biochemical characterization and tentative identification of bacterial isolates

The selected isolates were characterized and identified by gram staining [9,10] and standard biochemical tests [11, 12]. Several biochemical tests such as catalase test, indole test, urease test, oxidase test, citrate utilization test, nitrate reduction test and coagulase test were performed to categorize the isolates [13, 14]. In addition, methyl red test, gelatin liquefaction and fermentation of carbohydrates were performed according to described earlier [15].

Antibiotic Susceptibility Tests

The Kirby-Bauer method is a standardized method for antimicrobial resistance testing using to different antibiotic families [16]. In this method, Muller-Hinton agar plates were seeded by

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spread plate method with 0.1 mL of 24 h broth cultures of each isolate. A total of 26 antibiotic discs were used for susceptibility assay and 6 antibiotics were used for the each plate. After 24 h incubation, petriplates were observed for zone of inhibition around the antibiotic discs and the inhibition zone was measured in millimeter.

Data Analysis

The data collected in the study and the results of the laboratory investigations were analyzed using the statistical tools SPSS ver., 20.0. The P value < 0.05 was assumed to be important and the Chi-square test was conducted to measure the relation between the explanatory variables.

Results

Prevalence of urinary tract infection

During the study period, 320 samples of urine were collected, of which 142 samples were diabetic (44.38%). There were 76 cases of UTI in all diabetic patients and the prevalence of UTI was 53.5%.

Significant bacteriuria was confirmed from the urine of 72 non-diabetics patients with prevalence of 40.4%. The results showed prevalence of UTI is significantly higher in diabetic patients with *p*-value <0.05 than non-diabetics (Table 1). Meanwhile 43.2% men and 62.6% of women among diabetics patients developed UTI, which indicates that the incidence of UTI is considerably higher in diabetic women than men (p<0.02) (Table 2).

Table 1. Evaluation of prevalence of UTIs in diabetic and non-diabetic patients

	Prevalence of UTI in diabetic patients		***Prevalence of UTI in non-diabetics patients***		***Total***	***P value***
	Nos.	%	Nos.	%		
Patient with UTI	76	53.5	72	40.4	148	$X^2=5.429$,
Patient without UTI	66	46.5	106	59.5	172	***P=0.02***
Total	142	100	178	100	320	

Table 2. Assesment of UTI in diabetic patients according to the gender

	Prevalence of UTI in Male patients	***Prevalence of UTI in Female patients***	***Total***	***P value***

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	No	%	No	%		
Patient with UTI	29	43.2	47	62.6	76	X^2***=5.345,***
Patient without UTI	38	56.7	28	37.4	66	***P=0.021***
Total	67	100	75	100	142	

Bacterial isolates and antimicrobial susceptibility patterns

E. coli was the most common bacterium that was identified in 42 samples (28.4 %) in diabetic and in 43 samples (29.1%) in non-diabetic patients, while *Klebsiella* species showed the prevalence of 11 (7.4%) in diabetics and 8.0 (5.4%) in non-diabetics samples. *Enterococcus* sp. exhibited its occurrence in 9.0 (6.1%) in diabetic and 5.0 (3.4%) in non-diabetic patients. The incidence of *Candida* sp. was found 10 (7.4 %) in diabetic and 8.0 (5.5%) in non-diabetic people (Table 3).

Table 3. Prevalence of UTI in diabetic patients and non-diabetic patients according to gender

Micro organism	**Diabetics N=76**		**Non-diabetics N=72**	
	Male N=31	**Female N=45**	**Male N=31**	**Female N=41**
	N(%) =20.9	**N(%) =30.4**	**N(%) =20.9**	**N(%) =27.7**
E. coli	16(10.8)	26 (17.6)	18 (12.2)	25 (16.9)
Candida sp	4 (2.7)	6 (4.10	2 (1.4)	6 (4.1)
Klebsiella sp	4 (2.7)	7 (4.7)	4 (2.7)	4 (2.7)
Enterococcus sp	4 (2.7)	5 (3.4)	2 (1.4)	3(2.0)
Pseudomonas sp	2 (1.4)	1(0.7)	3 (2.0)	-
Proteus sp	1 (0.7)	-	-	1 (0.7)
Citrobacters sp		-	1(0.7)	1 (0.7)
Staphylocccs sp	-	-	1 (0.7)	

The isolated *Enterococcus* sp. from normal and diabetic patients was 100% sensitive to the linezolid, but resistant to the nitroforantoin, teicoplanine and vancomycine (Table 4). During the comparison of antibiotic sensitivity of *E. coli* strains which were isolated from the diabetic patients and non-diabetic one, the strains from the urine of diabetic people showed 100%

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resistance to the cefepime, co-trimoxazole, nitrofurantoin. On the other hand, most of the *E. coli* strains isolated from urine samples of diabetic patients were sensitive to piperacillin or tazobactam (73%) but strains isolated from non-diabetic patients were less (45%) sensitive to the same (Table 5). There was no significant difference of sensitivity to meropenem, fosfomycine to all strain of *E. coli*. *Klebsiella* sp. isolated from the urine culture of diabetic patients and non-diabetic patients was found to be 100% sensitive to the colistine, fosfomycine, ceftriaxone-sulbactam-EDTA but these were resistant to the cefepime, meropenem, ertapenem, piperacillin/tazobactam, norfloxacin as compare to those strains isolated from the urine of non-diabetic patients. The sensitivity to amikacin for *Klebsiella* was found equal in all the isolates. Further, *Pseudomonas* sp. that was isolated from the urine of diabetic and non-diabetic patients was 100% sensitive to colistine, fosfomycine and ceftriaxone-sulbactam-EDTA and resistant to cefepime, meropenem, etrapenem, piperacillin/tazobactam, norfloxacin, azithromycine and tobremycin as compared to those strains isolated from urine culture of non-diabetic patients. (Table 4).

Table 4. Antimicrobial susceptibility patterns of *Enterococcus* sp.

Enterococcus sp		**Antibiotics used for gram positive (*Enterococcus* sp.)**													
		LZ	**VA**	**NIT**	**NX**	**FO**	**AMP**	**COT**	**TEI**	**TE**	**RIF**	**A/S**	**C**	**HGC**	**TG**
Number of patients	**Total No=14**														
	Sensitivity Nos	14	7	7	0	1	2	1	5	4	0	2	2	0	1
	Sensitivity %	100	50	50	0	7.1	14.3	7.1	35.7	28.6	0.0	14.3	14.3	0.0	7.1
	Diabetic=9														
	Sensitivity Nos	9	5	4		1.0			4.0	2.0			2.0		
	Sensitivity %	100	35.7	28.5	0.0	7.1	0.0	0.0	28	14.3	0.0	0.0	14.3	0	0
	Non-diabetic=5														
	Sensitivity Nos	5	2	3	0	0	2	1	1	2	0	2	0	0	1
	Sensitivity %	100	14.3	21.4	0	0	14.3	7.1	7.1	14.3	0	14.3	0	0	7.1

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Bacteria	No. of patients	Antibiotic susceptibility for gram negative bacteria																			
		MRP	ETP	CL	AK	CAZ	FO	PIT	CTX	CFS	CSE	CTR	NX	AMC	CXM	TGC	IPM	CFM	OF	CZ
E. coli	DM=42	%	70.7	39.0	100	70.7	4.9	78.0	73.2	4.9	41.5	100	7.3	4.9	4.9	0.0	41.5	7.3	0.0	2.4	4.9
	NDM=43	%	63.6	43.2	100.0	75.0	9.1	88.6	45.5	9.1	45.5	100.0	4.5	4.5	11.4	11.4	45.5	6.8	9.1	6.8	2.3
Klebsiella sp	DM= 11	%	72.7	27.3	100	18.2	0.0	90.9	9.1	0.0	9.1	100	0.0	9.1	0.0	0.0	0.0	18.2	0.0	9.1	9.1
	NDM =8	%	0	12.5	100	12.5	12.5	37.5	25	12.5	25	100	0	0	0	0	25	0	0	0	-12.5
Psoudomona sp	DM=3	%	33.3	33.3	100	66.7	33.3	100	33.3	0	3.3	100	0	33.3	33.3	0	0	33.3	0	33.3	0
	NDM=3	%	66.7	66.7	100.0	66.7	0	100	66.7	0	0	100	0	66.7	33.3	0	33.3	33.3	0	33.3	0
Proteus sp	DM=1	%	100	0	100	0	100	100	0	0	100	0	0	0	0	0	0	0	0	0	0
	NDM =1	%	100	100	0	0	0	100	0	0	100	0	0	0	0	0	0	0	0	0	0

Table 5. A**ntimicrobial susceptibility patterns of gram negative bacterial isolates**

Discussion

No equality was found in the occurrence of bacterial species present in urine samples of diabetic and non-diabetic patients. In diabetic and non-diabetic samples, *E. coli* was in higher incidence than other UTI bacterial pathogens. It was observed that *E. coli* is responsible for high proportion (57.5%) of UTI in both diabetic (28.4%) and non-diabetic (29.1%) patients. Various serological, epidemiological and bacteriological studies have shown that uro pathogenic *E. coli* is the pathogen most frequently associated with UTIs [17]. The prevalence of UTI was observed more

ISSN:0971-1260
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in females than males. Female patients exhibited a higher percentage of UTI bacteria, 62.6% for diabetic females. It may be attributed to a short space of urethra and the closed vagina opening to the anal orifice [18-21].

Enterobacteriaceae species exhibited a small percentage of infected urine samples; 6.1% in diabetics and 3.4% in non-diabetics patients. It can be related with the pathogenicity due to presence of capsule [22]. The prevalence of Candida sp. was 7.4% in diabetic patients and 5.5% in non-diabetic patients.

In order to evaluate efficient antibiotics for the treatment of the patients at initial stage of UTI, the isolates were treated with different antibiotics. Colinstin and ceftriaxone-sublactam- EDTA were inhibiting the most isolated bacterial species in both diabetic and non-diabetic patients with UTIs. Most of the gram-negative bacteria were having a good susceptibility to amikacin, piperacillin/tazobactam, cefoperazone/sulbactam and carbapenems while Gram-positive bacteria were mostly sensitive to nitrofurantoin, linezolid and vancomycin. Multi-drug resistance is one of the main public health issues and its rate is increasing and this is likely to increase the load of managing urinary tract infection among diabetic patients. Among *E. coli* strains, 3 (2.9%) were evaluated as MDR (Multi drug resistant) and 80 (94.1%) were ESBL (Extended spectrum beta-lactamase). Also presence of ESBL among *Klebsiella* sp was 19 (100 %), which shows very high ESBL resistant rates. It is very necessary to find new antimicrobial and therapeutic agents having high efficacy with no side effect and low cost.

Conclusions

The occurrence of urinary tract infection is significantly higher in diabetic patients than non-diabetic patients and in women than men in both diabetic and non-diabetic groups. It is concluded that the isolate, *E. coli* was the most diverse bacterial species from the diabetic as well as non-diabetic patients with UTI. MDR strains of various pathogens are being increasing with the time. Further studies in this topic with wider sample size are recommended to validate the results found from this study.

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